

Short communication

A facile and improved synthesis of sildenafil (Viagra®) analogs through solid support microwave irradiation possessing tyrosinase inhibitory potential, their conformational analysis and molecular dynamics simulation studies

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Summary

Herein, the synthesis of some analogs of sildenafil (Viagra®) (**21**) is described, employing MW irradiations in key steps such as, S_NAr reaction on important precursor bromopyrazole (**7**). Compound **7** was synthesized by the bromination followed by the amidation of readily available 1-methyl-3-propyl-1*H*-pyrazole-5-carboxylic acid (**5**). Compounds **9** and **10** were obtained as S_NAr reaction products, apparently through the proposed dipolar high-energy transition states TS-1 and TS-2 under MW irradiation, respectively. In contrast, conventional heating failed to produce similar results, even after prolonged heating. Compound **10**, upon chlorosulfonation followed by the coupling of various nucleophiles, yielded a series of compounds **12–20** as analogs of sildenafil (**21**). Compounds **12–21** were subjected to tyrosinase inhibition studies and SAR studies were carried out. This study reflected that the inhibition was enhanced with increase of carbon chain. In case of the compound **17**, the –OH group was replaced with –CH₂–CH₂–OH with a resulting increase in inhibition against tyrosinase. Compound **17** was found to be more potent than the potent reference inhibitor LM and KA. The 2D and 3D hydrogen bonding descriptors that help to study QSPR were also calculated. Energetically most stable conformations of these compounds were analyzed. Their kinetic, potential and total energies were also calculated through MD simulation.

Abbreviations: AMPA, α -amino-3-hydroxy-5-methyl isoxazole-4-propionic acid; cGMP, cyclic guanosine monophosphate; EIMS, electron impact mass spectra; HYBOT, hydrogen bond thermodynamics; KA, kojic acid; LM, L-mimosine; MD, molecular dynamic; MED, male erectile dysfunction; SAR, structure activity relationship; MM, molecular mechanics; MMFF, molecular mechanics force field; MW, microwave; NMR, nuclear magnetic resonance; PDE5, phosphodiesterase type 5; PPO, polyphenol oxidase; QSPR, quantitative structure properties relationships; RMS, root mean square; SEM, standard error of the mean; S_NAr, aromatic nucleophilic substitution reaction; TS, transition state.

Introduction

Our research group believes that known therapeutic agents are not only effective for their known uses, but they may also be effective to cure other diseases. Even when a therapeutic agent causes unpleasant side effects when targeting a specific disease, it is also possible that the same therapeutic agent could exhibit other beneficial and useful effects for other diseases. In recent years, we have prepared a variety of known classes of compounds through improved synthetic methodologies for their random screening against a variety of new biological targets and the

identification of new lead compounds against new diseases [1].

During the course of these efforts, we decided to search alternate biological activities of sildenafil (Viagra®) [2] and related analogs apart from its known orally effective properties for the treatment of MED. Originally the citrate formulation of sildenafil was discovered to be an effective PDE5 inhibitor [3].

Herein we report our research on sildenafil (**21**) and its analogs (**12–20**) by using new emerging MW irradiation technology in our synthetic scheme as potent tyrosinase inhibitor along with the conformation analysis and molecular dynamic

simulation studies. Sildenafil [4] is a potent, reversible and selective PDE5 inhibitor that blocks cGMP hydrolysis effectively ($K_i = 3$ nM). PDE5 is the predominant cGMP-hydrolyzing enzyme present in the corpus cavernosum, the smooth muscle in the penis, which helps to control vascular tone. Despite the efficacy of sildenafil as a treatment for MED, there are some notable drawbacks associated with its use, which include headache (16%), facial flushing (10%), dyspepsia (7%) and visual disturbances (3%) [2]. Some of these side effects are thought to be due to non-specific inhibition of other PDEs, specifically PDE1 and PDE6 [5–6].

Solvent-free reactions [7] provide ease to synthetic transformations. This cuts down the hazards of high-pressure development, which could prevent or complicate the production of the reaction on large scale. Conventional heating has certain drawbacks in solid support synthesis due to poor thermal conductivity. MW irradiation provides uniformity of temperature in solid support reactions [8]. S_NAr reactions are of importance due to their use in industrial chemistry including the synthesis of *N*-alkyl aminoheterocycles [9], bioactive diarylethers [10], and *N,N*-disubstituted-5-aminothiophene-2-carboxaldehyde [10–11]. This type of reaction has also been used as a tool for making AMPA receptor antagonists, allowing the functionalization of the pyrrole nucleus [12] and the construction of the quinoxalinedione framework [13], as well as in synthesis of labeled 2'-deoxyinosine and 2'-deoxyadenosine [14]. Various catalysts [9–11, 15–16] are used to carry out S_NAr reactions, which otherwise require invariably higher temperature, toxic metal ions, excess reagents, and are not environmental friendly.

We have worked on synthesis of some sildenafil's analogs *via* microwave-assisted synthesis, utilizing its best attributes such as heating output, short reaction time, inexpensive materials, and mild reaction conditions. The tyrosinase inhibitory study on this class of compounds opens up a door for the identification of novel lead candidates.

Tyrosinase (EC 1.14.18.1), also known as PPO, is a multifunctional copper-containing enzyme widely distributed in plants and animals. It catalyzes the *o*-hydroxylation of monophenols and also the oxidation of *o*-diphenols to *o*-quinones. This enzyme is known to play a key role in the biosynthesis of melanin in plants and animals. Therefore, tyrosinase inhibitors are clinically useful for the treatment of some dermatological disorders associated with melanin hyper pigmentation and also for their use in cosmetics. In addition, tyrosinase is known to be involved in the molting process of insect and adhesion of marine organisms [17]. This enzyme has also been reported to be involved in the generation of *o*-diphenols and quinines for pigmentation, wound healing, parasite encapsulation, and sclerotization. This enzyme is currently used to control insect pests. In the food industry, tyrosinase is responsible for the enzymatic browning reactions in damaged fruits during post-harvest handling and processing. Control of enzymatic browning during processing is important in fruit pulp manufacturing. In addition, tyrosinase inhibitors are becoming important constituents of

cosmetic products that relate to hyperpigmentation. Therefore, there is a concerted need to search for new tyrosinase inhibitors from a variety of sources [18].

Results and Discussion

Chemistry

The starting materials, 2,4-dioxoheptanoate (**3**) [4, 19], methyl-5-propyl-2*H*-pyrazole-3-carboxylate (**4**) [4, 20–22], 1-methyl-3-propyl-5-pyrazolecarboxylic acid (**5**) [4, 20–22], chlorosulfonyl derivative of 5-(2-ethoxyphenyl)-1-methyl-3-propyl-1,6-dihydro-7 *H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**11**) and sildenafil (**21**), were prepared by using known literature procedures [6, 23]. As shown in Figure 1, the synthesis commenced with the bromination at activated 4-position of 1-methyl-3-propyl-5-pyrazolecarboxylic acid (**5**) to the corresponding 4-bromo-1-methyl-3-propyl-5-pyrazolecarboxylic acid (**6**) (see the experimental section). Compound **6** was converted into the respective acid chloride in refluxing $SOCl_2$. The resulting acid chloride was converted into the respective amide upon treatment with aqueous NH_4OH at room temperature, followed by the MW assisted S_NAr reaction on activated C-4 position of 4-bromo-1-methyl-3-propyl-5-pyrazolecarboxamide (**7**) through the nucleophilic attack of $-NH_2$ group of 2-ethoxybenzamide (**8**) giving rise to the products identified as compounds **9** and **10** as key products. The reaction was performed on basic alumina (7 min, MW) in dry media to trap the HBr formed. However, under conventional heating, reaction of compounds **7** with **8** by using potassium *t*-butoxide in *t*-butanol, yields only the traces of compound **9**. The reaction proceeds through the transition states **TS-1** and **TS-2** Figure 2 to afford compounds **9** (20%) and **10** (75%) along with some undetectable side products (5%).

These high-energy TS can be achieved under MW irradiation by acquiring the certain vibrational levels of the molecules, by exciting them through a speedy MW heating, which cannot be obtained sufficiently by the conventional heating even after 60 h (Table 1). The formation of compound **10** followed a proposed transition state (**TS-2**) through an intermediate **9** under MW. The formation of compound **9** provides an additional proof for the hypothetical pathway to achieve the compound **10**. Compound **9** was isolated by silica gel column chromatography (CH_2Cl_2 : EtOAc, 1:1) along with some other unidentified minor side products and was characterized by spectroscopic analysis. These side products may be due to decomposition of **10**, while prolonged heating (>7 min) results in the complete decomposition of the product (Table 1, Figure 3). The transformation of compound **9** into compound **10** was achieved by both MW and conventional heating in excellent yields (Table 1).

Compound **10** was converted into its chlorosulfonyl derivative **11** upon treating with $ClSO_3H$ following the literature procedure [4, 23]. Finally, compound **11** was treated with various nucleophiles (Figure 4) to afford compounds **12–20** as new derivatives of sildenafil (**21**). To the best of our

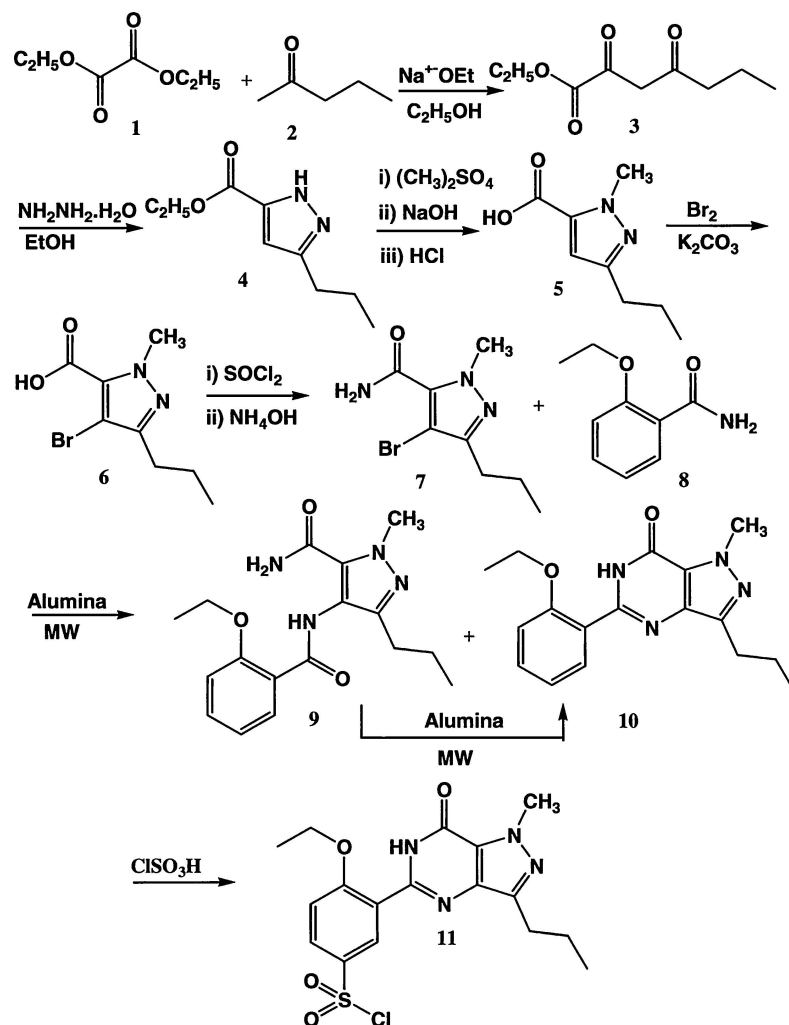


Figure 1. Synthetic route for compound 11.

knowledge, it is the first report of the synthesis of sildenafil's analogs using MW irradiation.

All newly synthesized compounds **12–21** and **11** were subjected to the tyrosinase inhibition assay. Tyrosinase inhibitory activities of the compounds **12–20**, as compared with the standard inhibitors are presented in Table 2. Out of these analogs, four exhibited mild to potent inhibition of the enzyme tyrosinase. Compounds **14** and **16** exhibited moderate to mild tyrosinase inhibition with their IC_{50} values of 19.95 and 54.43 μM , respectively. On the other hand, compounds **15** and **17** showed potent inhibition of tyrosinase with low IC_{50} values of 8.69 and 3.54 μM , respectively, comparable to standard tyrosinase inhibitors, like KA, 16.92 μM [24] and LM, 3.68 μM [25].

The compound **14**, where R is 2-methylpiperidine, showed good tyrosinase inhibitory activity with $\text{IC}_{50} = 19.95 \mu\text{M}$, whereas 3- and 4-methyl piperidinyl derivatives **12** and **13** were found to be inactive. The decrease in activity of compound **12** and **13** was apparently due to change of position of methyl group. In other words, the substitution of methyl group at position-2 is best fit for the activity and

may help in the interaction of the molecule with enzyme. The activity of compound **15** ($\text{IC}_{50} = 8.69 \mu\text{M}$) may be rationalised by its secondary amino function and its aromatic characters. Both of these effects may help to bind the molecule with the enzyme. The decrease in activity of compound **16** ($\text{IC}_{50} = 54.43 \mu\text{M}$) may be due to direct hydroxyl attachment with amino function, which may decrease the binding affinity of hydroxyl group with the enzyme [17]. The active most compound of the series was compound **17**, which was the ethanolamine derivative of compound **11**. The enhanced activity of compound **17** ($\text{IC}_{50} = 3.54 \mu\text{M}$) compared to the compound **16** may be explained on the basis of the electronegativity differences in carbon and oxygen. In compound **16**, the nitrogen atom was directly attached with oxygen, which causes lowering the electron density on nitrogen atom, which may bind with the copper of the enzyme to inhibit its unfavorable activities. However, in case of compound **17**, oxygen is present two carbons away from the nitrogen, which may additionally enhance the ability to bind the molecule to the enzyme by either the oxygen or nitrogen of molecule [17].

Table 1. Tabular representation of MW assisted formation of compounds 9 and 10, as compared with the conventional heating

S. No.	Starting Material	Time		Products		Yield%	
		MW	Δ	MW	Δ	MW	Δ
1	Comp 7 + Comp 8	9 min	60 h	a	9 , –	Decomp.	Traces, –
2	Comp 7 + Comp 8	7 min	60 h	9 , 10 , ^a	9 , –	20, 75, 5 ^a	Traces, –
3	Comp 7 + Comp 8	5 min	40 h	9 , 10 , ^a	9 , –	30, 60, 5 ^a	Traces, –
4	Comp 7 + Comp 8	4 min	20 h	9 , 10 , ^b	–, –	35, 45, 20 ^b	–, –
5	Comp 9	1 min	10 h	10	10	100	85

MW = Microwave heating; Δ = Conventional heating. ^aDecomposed or side products;

^bStarting materials, – = no product

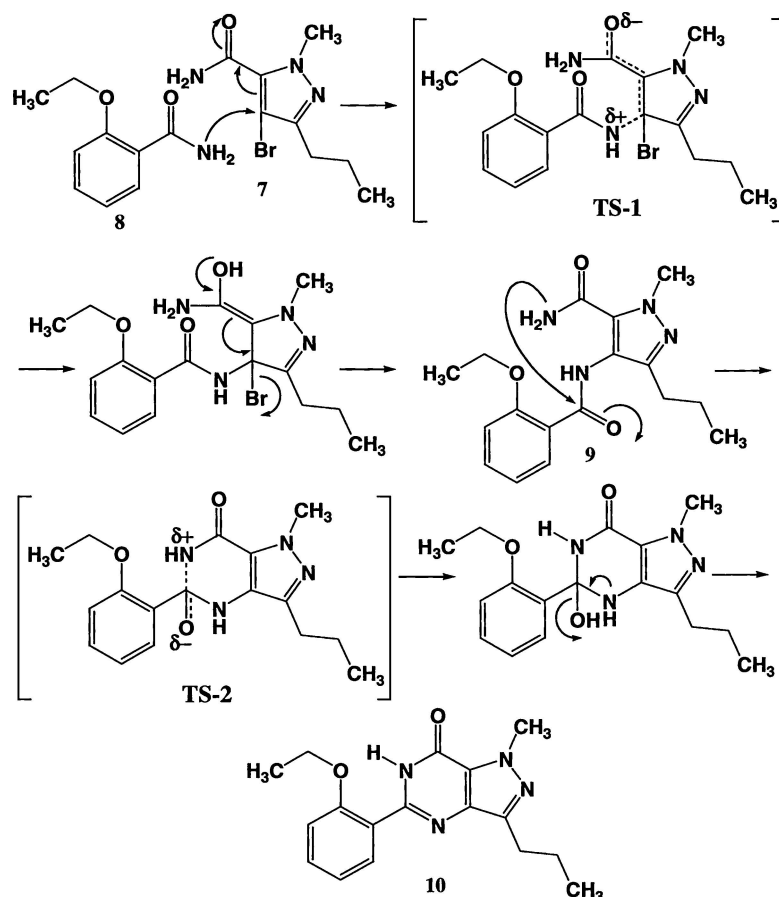


Figure 2. Mechanistic pathway for compound 10.

The most energetically stable conformational forms of sildenafil and its four analogs, which have exhibited tyrosinase inhibition, are shown in Figure 5. The energy minimization experiments have been carried with the help of the Block-Diagonal Newton-Raphson algorithm at the RMS gradients of 0.1 Kcal/(Å mol) for different cycles *in vacuo* depending on the specific molecule. Their kinetic, potential, and total energies were calculated through the MD, MM + MMFF simulation experiments, which are graphically presented in the Figures 6–10 for sildenafil (**21**) and compounds **14–17**, respectively. The simulations were run *in vacuo* at temper-

atures of 300 K, using the starting temperature at 0 K, and final temperature step was 20 K.

General experiment

Melting points were determined by a Büchi 434 melting point apparatus and are uncorrected. NMR spectroscopy was performed on a Bruker AVANCE 300 and 500 MHz. IR spectra were recorded on JASCO IR-A-302 Spectrophotometer. EIMS were recorded on a FINNIGAN MAT-311A Germany.

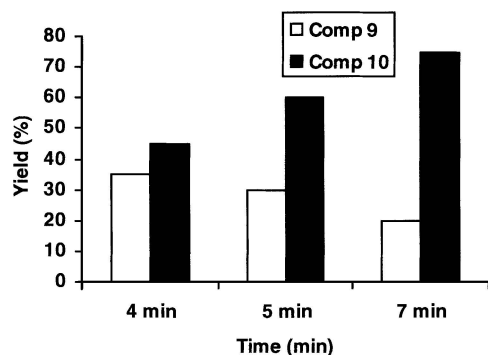


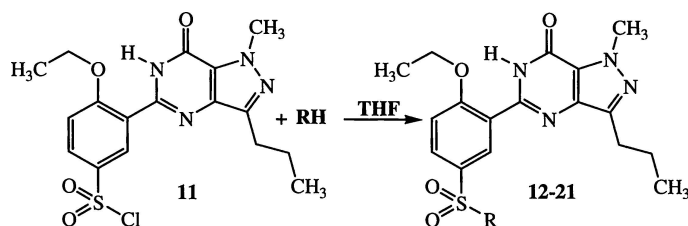
Figure 3. Graphical representation of time effects on yields of compounds 9 and 10 under MW irradiation.

MW assisted reactions were performed using 900 W, 2,450 MHz (LG Domestic Microwave Appliance) oven. TLC was performed on pre-coated TLC (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by iodine vapors, UV light at 254 and 365 nm, Ceric sulphate solution followed by heating and Dragendroff's reagent. Column chromatography was performed on silica gel

(Kieselgel 60, 254 E. Merck, Germany 230–400 mesh) using commercial grade solvents. Diethyloxalate, dimethylsulfate, bromine and 2-pentanone were purchased from Merck. The tetrahydrofuran and methanol used in reactions were of analytical reagent grade (E. Merck) and dried before use whenever necessary by using standard protocols.

Tyrosinase inhibition assay

Tyrosinase inhibition assays were performed in 96-well micro-plate format using SpectraMax[®] 340 (Molecular Devices, CA, USA) microtiter-plate reader according to the developed method earlier described by Hearing [26]. Briefly, all the compounds were dissolved in DMSO to a concentration of 2.5%. 30 Units mushroom tyrosinase (28 nM) were first pre-incubated with the compounds, in 50 nM Na-phosphate buffer (pH 6.8) for 10 min at 25 °C. Then the L-DOPA (0.5 mM) was added to the reaction mixture and the enzyme reaction was monitored by measuring the change in absorbance at 475 nm (at 37 °C) of the formation of the DOPACHROME for 10 min. The percent inhibition of the enzyme and IC₅₀ values



Compounds	R	Yield (%)
12		94
13		93
14		86
15		95
16		78
17		86
18		88
19		75
20		70
21		91

Figure 4. Synthetic scheme for preparation of compounds 12–21.

Table 2. Tyrosinase inhibitory activities of the compounds, as compared to the standard inhibitors

Compounds	IC ₅₀ (Mean \pm S.E.M. ^a) (in μ M)
11	NA ^c
12	NA ^c
13	NA ^c
14	19.95 \pm 0.19047
15	8.69 \pm 0.01044
16	54.43 \pm 0.05372
17	3.54 \pm 0.00067
18	NA ^c
19	NA ^c
20	NA ^c
21	NA ^c
Kojic acid ^b	16.67 \pm 0.5190
L-Mimosine ^b	3.68 \pm 0.02234

^aSEM is the standard error of the mean;

^bstandard inhibitors (KA and LM) of the enzyme tyrosinase; ^cnot active against tyrosinase.

of the active compounds were calculated using a program developed with Java and Macro Excel[®] 2000 (Microsoft Corp., USA) for this purpose. The following equation was used:

$$\text{Percent inhibition} = [\text{ABS}_{\text{Blank}} - \text{ABS}_{\text{Sample}} / \text{ABS}_{\text{Blank}}] \times 100$$

Here $\text{ABS}_{\text{Blank}}$ and $\text{ABS}_{\text{Sample}}$ are the absorbance for the blank and samples, respectively. All the studies have been carried out at least in triplicate and the results here represent the mean \pm SEM. All the reagents, enzyme, substrate, and reference compounds, were purchased from Sigma Chem. Co., MO, USA.

Molecular modeling studies

Energy minimization

The 2D structures of the molecules have been drawn with CS ChemDraw[®] Pro version 6.0 (CambridgeSoft Corporation), which has been further changed into 3D forms and hydrogen atoms were added with the help of HyperChem[®] Professional version 7.1 (HyperCube Inc., Gainesville, FL, USA).

Energy minimization experiments were carried out using HyperChem[®] employing the Block-Diagonal Newton-Raphson algorithm at the RMS gradients of 0.1 Kcal/(Å mol) for different cycles *in vacuo*. For rendering and refinement of the 3D structures, Persistence of Vision (tm) Ray-Tracer (Pov-Ray[®]) MS windows XP[®] version program was used [27–28].

Molecular Dynamics (MD)

The MD, MM+, MMFF experiments were performed using HyperChem[®] Professional version 7.1 (HyperCube Inc., Gainesville, FL, USA) on MS windows XP[®]. Electrostatic bond dipoles have been taken for the option of MM+ simulations. In these experiments, total run time was fixed for 1.0 pico sec, heating and cooling times were 0.5 pico sec, and step size was 0.001 pico sec. The simulations were run *in vacuo* at temperatures 300 K with starting and final temperature of 0 K and temperature step was 20 K. For the force field analysis bond angle, torsion, non-bonded, electrostatic, and hydrogen bonded, taken as the components for the MM+ simulations [27–28].

Hydrogen bonding properties

Hydrogen bonding plays a critical role in functional biology of living organisms. The hydrogen bonding properties of Sildenafil and its analogs were studied using the software 2D and 3D HYBOT, where different descriptors parameters were calculated [29–30].

4-Bromo-1-methyl-3-propyl-1H-pyrazole-5-carboxylic acid (6)

To a solution of **5** (0.168 g, 1 mmole) and K₂CO₃ (0.414 g, 3 mmole) in CH₂Cl₂ (20 mL) at room temperature, Br₂ (3 mmole), was added under darkness. After 6 h at room temperature, the reaction was quenched with 1 M Na₂S₂O₃ (30 mL), extracted with CH₂Cl₂ (3 \times 25 mL), dried over Na₂SO₄, and evaporated under reduced pressure to dryness. The residue was chromatographed (CH₂Cl₂: EtOAc, 1:1) and gave the title compound as a brown amorphous powder 0.148 g (Yield = 88%). Mp = 112–113 °C; R_f = 0.46 (EtOAc: CH₂Cl₂, 6:4); [Found: C (38.92), H (4.47), N (11.33). C₈H₁₁BrN₂O₂ requires C (38.89), H (4.49), N (11.34)]; IR (KBr): ν_{max} 2933, 2873 (O–H, C–H br. str. overlapped), 1709 (C=O), 1465 (C=C str.), 1534 (CH₂ bend.), 1390, 1362 (CH₃ bend.), 1240 (C–N str.), 610 (C–Br str.) cm^{–1}; ¹H–NMR (MeOD-*d*₄, 300 MHz): δ 0.98 (t, J = 7.2 Hz, 3H, CH₂–CH₂–CH₃), 1.67 (m, 2H, CH₂–CH₂–CH₃), 2.81 (dd, J = 7.2 Hz, 7.8 Hz, 2H, CH₂–CH₂–CH₃), 3.92 (s, 3H, N1–CH₃); EIMS: m/z 249 (M⁺, 18), 247 (20), 233 (18), 203 (60), 191 (8), 189 (10), 168 (100), 154 (10), 141 (14), 124 (30), 97 (11), 44 (5%).

4-Bromo-1-methyl-3-propyl-1H-pyrazole-5-carboxamide (7)

Compound **6** (0.247 g, 1 mmole) was refluxed with SOCl₂ (15 mL) for 2 h (oil bath). Excess of SOCl₂ was distilled under reduced pressure and the crude acid chloride was added slowly to a cooled (\approx 0 °C) aqueous NH₄OH (20 mL, 25%). The reaction mixture was stirred for 2 h at room temperature. Cold water (50 mL) was then added. The precipitated solid product **7** was collected by suction filtration and dried to obtain light yellow powder 0.21 g (85%). Mp = 133–134 °C; R_f = 0.42 (EtOAc: CH₂Cl₂, 7:3); [Found: C (39.01), H (4.89), N (17.10). C₈H₁₂BrN₃O

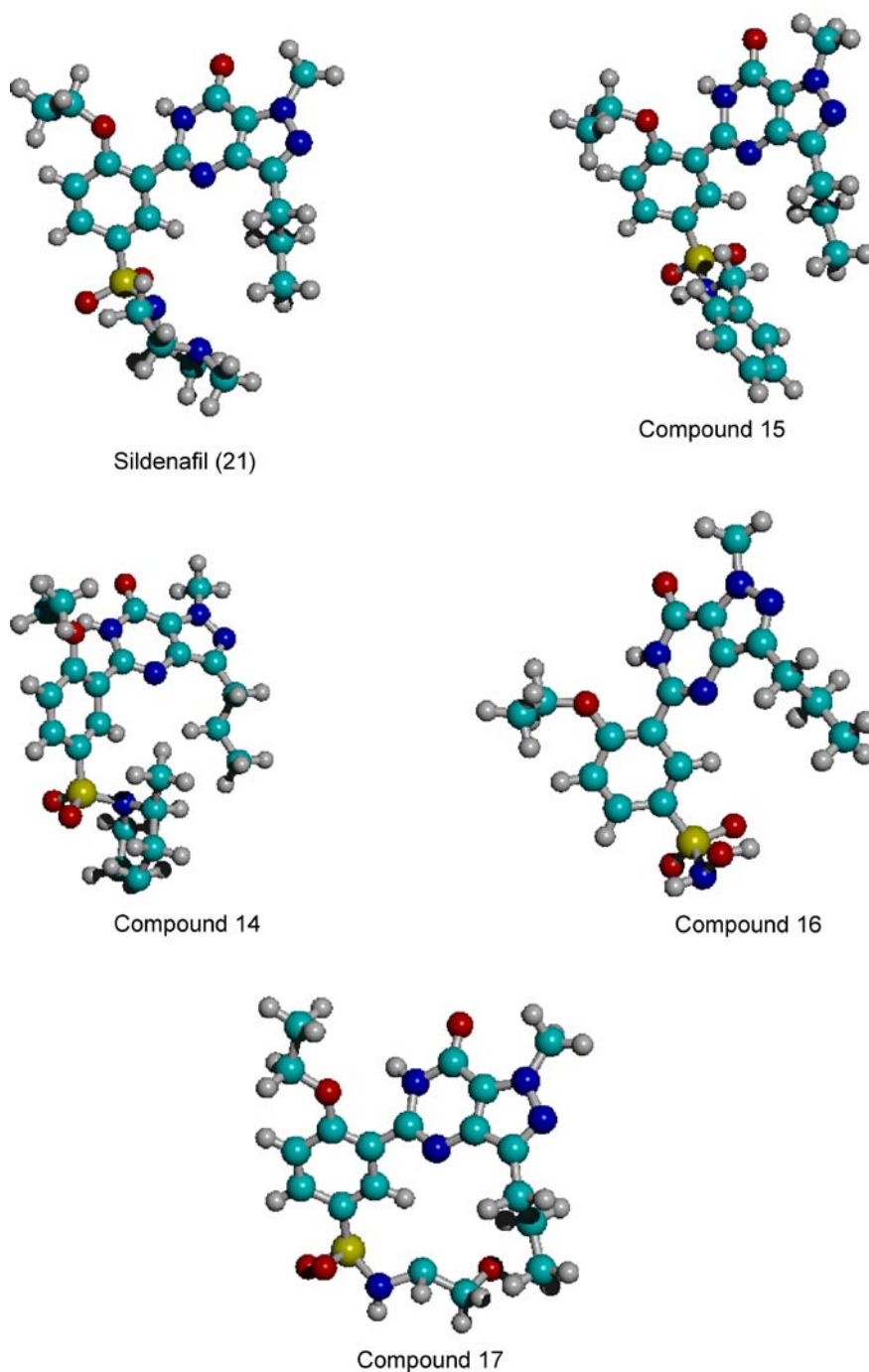


Figure 5. Energy minimized structures of sildenafil and its four analogs showing mild to potent tyrosinase inhibition.

requires C (39.04), H (4.91), N (17.07)]; IR (KBr): ν_{\max} 3400 (amide-NH₂), 2873 (C–H str.), 1710 (C=O), 1460 (C=C str.), 1532 (CH₂ bend.), 1388, 1359 (CH₃ bend.), 1245 (C–N str.), 617 (C–Br str.) cm⁻¹; ¹H-NMR (CDCl₃, 300 MHz): δ 0.96 (t, J = 7.4 Hz, 3H, CH₂–CH₂–CH₃), 1.68 (m, 2H, CH₂–CH₂–CH₃), 2.80 (dd, J = 7.1 Hz, 7.6 Hz, 2H, CH₂–CH₂–CH₃), 2.85 (br s, 2H, –NH₂), 3.92 (s, 3H, N1-CH₃); EIMS: m/z 248 (M⁺, 22), 246 (25), 231 (13), 203 (35),

189 (10), 167 (100), 141 (19), 110 (48), 97 (11), 71 (16), 44 (5%).

4-[(2-Ethoxybenzoyl)amino]-1-methyl-3-propyl-1H-pyrazole-5-carboxamide (9)

4-Bromo-1-methyl-3-propyl-1H-pyrazole-5-carboxamide (7) (0.246 g, 1 mmol) and 2-ethoxybenzamide (8) (0.165, 1 mmol) was adsorbed on basic alumina. The reaction

mixture enclosed in closed vessel of Teflon[®] was irradiated inside the MW oven for 6–7 min. at 2,450 MHz. The reaction mixture was cooled to room temperature and eluted with CH₂Cl₂ (3 × 15 mL). The eluent was evaporated to dryness and subjected to the column chromatography (CH₂Cl₂: EtOAc, 1:1) to afford compound **9** (0.05 g, 20%) and compound **10** (0.185 g, 75%) along with some undetectable side products (5%). The structure of the compound **9** was determined by the spectroscopic analysis were found in agreement with the reported literature values [4, 23].

5-(2-Ethoxyphenyl)-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (10)

Microwave irradiation method:

- Compound **10**, 0.185 g (75% yields), was obtained as one of the reaction products of compounds **7** and **8** (see procedure for compound **9**)
- After purification, compound **9** (0.05 g, 0.151 mmoles) was again subjected to MW irradiation for 1 min. following the same reaction protocol as followed for the synthesis of compound **9** to afford compound **10**, 0.047 g (100% yield).

Conventional heating method:

Potassium *t*-butoxide (0.224 g, 2 mmoles) was added to a stirred suspension of amide **7** (0.33 g, 1 mmoles) in *t*-butanol (10 mL). The resultant mixture was refluxed for 10 h at 95 °C (oil bath), and then allowed to cool at room temperature. Water (15 mL) was added, the solution was neutralized with HCl (5%) to pH ≈ 7, and cooled to about 5–10 °C. The precipitated solid product was collected by suction filtration, washed with cold water, and dried. This afforded the title compound in 0.281 g (85% yield).

Compound **10** showed the same spectroscopic and physical data as reported earlier [4, 23].

Chlorosulfonyl derivative of 5-(2-Ethoxyphenyl)-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (11)

The title compound was obtained in 86% yield (0.112 g) by following the literature procedure [4, 23].

General procedure for the preparation of compounds 12–20

The sulfonyl chloride derivative **11** (1 mM) was dissolved in THF (35 mL), and treated with a solution of various amines and thiols (5 mmoles) in THF (10 mL, Figure 5). The resulting mixture was stirred at room temperature for 2.5 h, THF was then removed, and the residue was treated with cold H₂O (40 mL). The resulting precipitates were filtered under suction, washed with little ice-cold water, drained to dryness, and recrystallized from aqueous EtOH.

1[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperidine (12)

White amorphous powder; Yield: 0.089 g (94%); Mp = 178–179 °C; *R_f* = 0.51 (EtOAc: CH₂Cl₂, 2:8); [Found: C (58.36), H (6.58), N (14.81). C₂₃H₃₁N₅O₄S requires C (58.33), H (6.60), N (14.79)]; IR (KBr): ν_{\max} 3290 (N–H str.), 2933, 2873 (C–H str.), 1709 (C=O), 1601 (N–H bend.), 1578, 1465 (Ar C=C str.), 1534 (CH₂ bend.), 1390, 1362 (CH₃ bend.), 1335 (S=O asym. str.), 1244 (C–N str.), 1168 (C–O) cm^{−1}; ¹H-NMR (CDCl₃, 500 MHz): δ 0.90 (br d, *J* = 4.5 Hz, 3H, 4-CH₃-piperidiny), 1.00 (t, *J* = 7.3 Hz, 3H, CH₂–CH₂–CH₃), 1.55 (br s, 4H, C-3''H/C-5''H), 1.62 (t, *J* = 6.9 Hz, 3H, O–CH₂–CH₃), 1.66 (br s, 1H, C-4''H), 1.84 (m, 2H, CH₂–CH₂–CH₃), 2.32 (br t, 4H, C-2''H/C-6''H), 2.91 (dd, *J* = 7.1 Hz, 7.8 Hz, 2H, CH₂–CH₂–CH₃), 4.25 (s, 3H, N1-CH₃), 4.35 (dd, *J* = 6.9 Hz, 13.9 Hz, 2H, O–CH₂–CH₃), 7.12 (d, *J* = 8.7 Hz, 1H, C-3'H), 7.83 (dd, *J* = 2.2 Hz, *J* = 8.7 Hz, 1H, C-4'H), 8.81 (d, *J* = 2.2 Hz, 1H, C6'-H), 10.79 (s, 1H, N6-H). EIMS: *m/z* 473 (M⁺, 93), 457 (32), 445 (77), 376 (10), 312 (41), 283 (34), 268 (11), 253 (15), 239 (20), 193 (27), 166 (47), 136 (26), 98 (100), 73 (15), 56 (22%).

1[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-3-methylpiperidine (13)

White amorphous powder; Yield: 0.078 g (93%); Mp = 165–166 °C; *R_f* = 0.52 (EtOAc: CH₂Cl₂, 2:8); [Found: C (58.36), H (6.57), N (14.80). C₂₃H₃₁N₅O₄S requires C (58.33), H (6.60), N (14.79)]; IR (KBr): ν_{\max} 3292 (N–H str.), 2932, 2875 (C–H str.), 1709 (C=O), 1600 (N–H bend.), 1578, 1468 (Ar C=C str.), 1533 (CH₂ bend.), 1391, 1362 (CH₃ bend.), 1335 (S=O asym. str.), 1243 (C–N str.), 1165 (C–O) cm^{−1}; ¹H-NMR (CDCl₃, 500 MHz): δ 0.87 (d, *J* = 6.5 Hz, 3H, 4-CH₃-piperidiny), 0.99 (t, *J* = 7.4 Hz, 3H, CH₂–CH₂–CH₃), 1.57 (br s, 2H, C4''-H), 1.57 (br s, 2H, C-4''H), 1.62 (t, *J* = 6.9 Hz, 3H, O–CH₂–CH₃), 1.69 (br s, 2H, C-5''H), 1.71 (br s, 1H, C-3''H), 1.84 (m, 2H, CH₂–CH₂–CH₃), 2.30 (br d, *J* = 11.3 Hz, 2H, C-2''H), 2.90 (d, *J* = 7.2 Hz, 7.7 Hz, 2H, CH₂–CH₂–CH₃), 3.6 (br d, *J* = 11.2 Hz, 2H, C-6''H), 4.25 (s, 3H, N1-CH₃), 4.35 (dd, *J* = 6.9 Hz, 13.9 Hz, 2H, O–CH₂–CH₃), 7.12 (d, *J* = 8.7 Hz, 1H, C-3'H), 7.83 (dd, *J* = 2.3 Hz, 8.7 Hz, 1H, C-4'H), 8.80 (d, *J* = 2.3 Hz, 1H, C-6'H), 10.79 (s, 1H, N6-H). EIMS: *m/z* 473 (M⁺, 86), 457 (31), 445 (79), 376 (11), 312 (35), 283 (33), 268 (10), 253 (12), 239 (18), 193 (26), 166 (42), 136 (34), 98 (100), 73 (15), 56 (17%).

1[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-2-methylpiperidine (14)

White amorphous powder; Yield: 0.081 g (86%); Mp = 154–155 °C; *R_f* = 0.50 (EtOAc: CH₂Cl₂, 2:8); [Found: C (58.33), H (6.61), N (14.78). C₂₃H₃₁N₅O₄S requires C (58.33), H (6.60), N (14.79)]; IR (KBr): ν_{\max} 3287 (N–H str.),

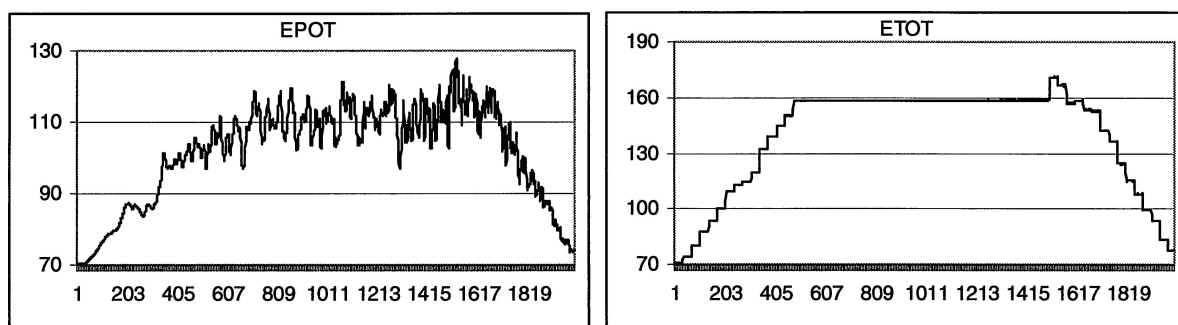


Figure 6. Energy [potential (EPOT) and total (ETOT)] calculations plots [time (X, in pico sec.) vs. energy (Y, Kcal/mol)] of molecular dynamic (MD) of sildenafil (21).

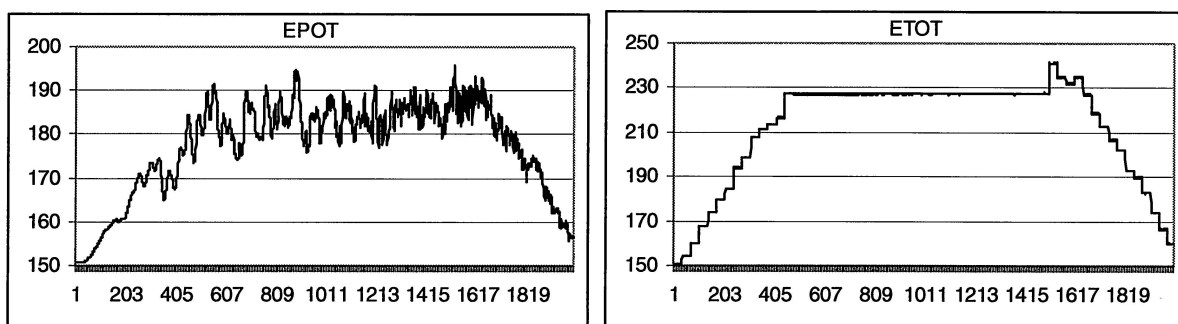


Figure 7. Energy [potential (EPOT) and total (ETOT)] calculations plots [time (X, in pico sec.) vs. energy (Y, Kcal/mol)] of molecular dynamic (MD) of compound 14.

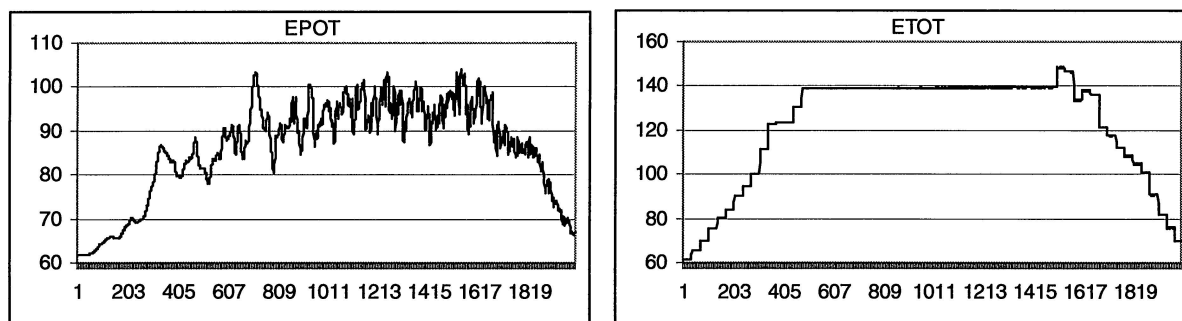


Figure 8. Energy [potential (EPOT) and total (ETOT)] calculations plots [time (X, in pico sec.) vs. energy (Y, Kcal/mol)] of molecular dynamic (MD) of compound 15.

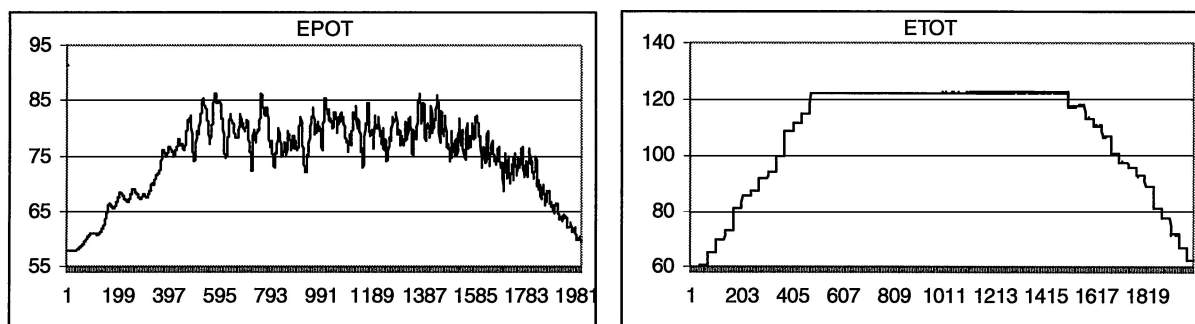


Figure 9. Energy [potential (EPOT) and total (ETOT)] calculations plots [time (X, in pico sec.) vs. energy (Y, Kcal/mol)] of molecular dynamic (MD) of compound 16.

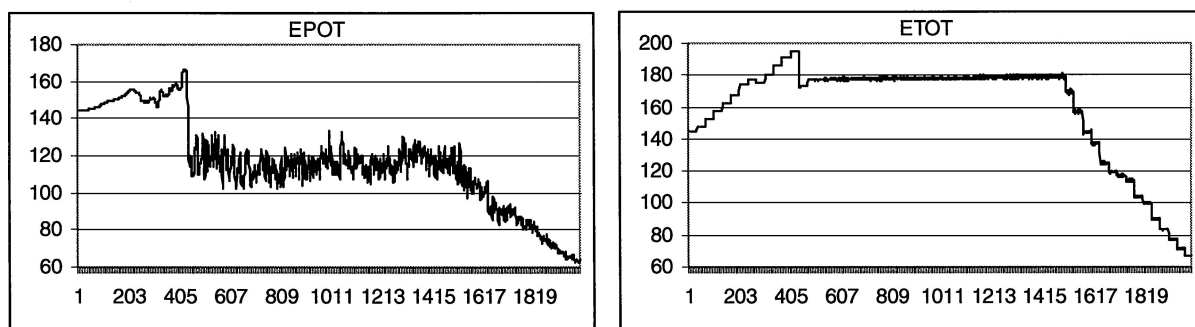


Figure 10. Energy [potential (EPOT) and total (ETOT)] calculations plots [time (X, in pico sec.) vs. energy (Y, Kcal/mol)] of molecular dynamic (MD) of compound 17.

2935, 2870 (C–H str.), 1711 (C=O), 1600 (N–H bend.), 1578, 1467 (Ar C=C str.), 1533 (CH₂ bend.), 1391, 1362 (CH₃ bend.), 1333 (S=O asym. str.), 1242 (C–N str.), 1169 (C–O) cm⁻¹; ¹H-NMR (CDCl₃, 500 MHz): δ 0.99 (t, J = 7.3 Hz, 3H, CH₂–CH₂–CH₃), 1.15 (d, J = 6.9 Hz, 3H, 2-CH₃-piperidiny), 1.47 (br s, 2H, C-4''H), 1.57 (br s, 2H, C-5''H), 1.62 (t, J = 6.9 Hz, 3H, O–CH₂–CH₃), 1.83 (m, 2H, CH₂–CH₂–CH₃), 2.90 (dd, J = 7.0 Hz, 7.6 Hz, 2H, CH₂–CH₂–CH₃), 3.04 (br t, J = 12.7 Hz, 2H, C-6''H), 3.71 (dd, J = 2.7 Hz, 12.7 Hz, 2H, C-6''H), 4.25 (s, 3H, N1-CH₃), 4.34 (dd, J = 6.9 Hz, 13.9 Hz, 2H, O–CH₂–CH₃), 7.08 (d, J = 8.8 Hz, 1H, C-3'H), 7.91 (dd, J = 2.4 Hz, 8.8 Hz, 1H, C-4'H), 8.89 (d, J = 2.4 Hz, 1H, C-6'H), 10.85 (s, 1H, N6–H). EIMS: m/z 473 (M⁺, 66), 457 (29), 445 (68), 376 (17), 312 (29), 283 (30), 268 (16), 253 (11), 239 (21), 193 (27), 166 (37), 136 (31), 98 (100), 73 (18), 56 (19%).

1[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-benzylamine (15)

White amorphous powder; Yield: 0.093 g (95%); Mp = 189–190 °C; R_f = 0.46 (EtOAc: CH₂Cl₂, 1:9); [Found: C (59.83), H (5.66), N (14.53). C₂₄H₂₇N₅O₄S requires C (59.86), H (5.65), N (14.54)]; IR (KBr): ν_{\max} 3414, 3290 (N–H str.), 2958, 2870 (C–H str.), 1707 (C=O), 1600 (N–H bend.), 1578, 1485 (Ar C=C str.), 1531 (CH₂ bend.), 1390 (CH₃ bend.), 1335 (S=O asym. str.), 1245 (C–N str.), 1156 (C–O) cm⁻¹; ¹H-NMR (CDCl₃, 500 MHz): δ 0.89 (t, J = 7.3 Hz, 3H, CH₂–CH₂–CH₃), 1.47 (t, J = 6.9 Hz, 3H, O–CH₂–CH₃), 1.72 (m, 2H, CH₂–CH₂–CH₃), 2.81 (dd, J = 7.1 Hz, 7.6 Hz, 2H, CH₂–CH₂–CH₃), 4.03 (s, 2H, CH₂–Ar), 4.15 (s, 3H, N1-CH₃), 4.23 (dd, J = 6.9 Hz, J = 13.8 Hz, 2H, O–CH₂–CH₃), 7.01 (d, J = 8.8 Hz, 1H, C-3'H), 7.12 (br s, 5H, Ar–H), 7.81 (dd, J = 2.0 Hz, 8.7 Hz, 1H, C-4'H), 8.56 (d, J = 2.0 Hz, 1H, C-6'H), 10.58 (s, 1H, N6–H). EIMS: m/z 481 (M⁺, 99), 466 (33), 453 (72), 311 (12), 283 (13), 268 (9), 253 (14), 193 (19), 166 (54), 136 (44), 91 (100), 56 (11%).

1[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-hydroxylamine (16)

White amorphous powder; Yield: 0.083 g (78%); Mp = 135–136 °C; R_f = 0.55 (EtOAc: CH₂Cl₂, 3:8); [Found: C (50.14), H (5.21), N (17.20). C₁₇H₂₁N₅O₅S requires C (50.11), H (5.19), N (17.19)]; IR (KBr): ν_{\max} 3305 (N–H str.), 2958 (C–H str.), 1686 (C=O), 1601 (N–H bend.), 1581, 1488 (Ar C=C str.), 1533 (CH₂ bend.), 1392 (CH₃ bend.), 1333 (S=O asym. str.), 1245 (C–N str.), 1164 (C–O) cm⁻¹; ¹H-NMR (CDCl₃, 500 MHz): δ 0.90 (t, J = 7.3 Hz, 3H, CH₂–CH₂–CH₃), 1.47 (t, J = 6.9 Hz, 3H, O–CH₂–CH₃), 1.72 (m, 2H, CH₂–CH₂–CH₃), 2.80 (dd, J = 7.2 Hz, 7.8 Hz, 2H, CH₂–CH₂–CH₃), 4.15 (s, 3H, N1-CH₃), 4.23 (dd, J = 6.9 Hz, 13.8 Hz, 2H, O–CH₂–CH₃), 6.25 (br s, 1H, NH–OH), 7.05 (d, J = 8.8 Hz, 1H, C-3'H), 7.90 (dd, J = 2.0 Hz, 8.8 Hz, 1H, C-4'H), 8.59 (d, J = 2.0 Hz, 1H, C-6'H), 10.67 (s, 1H, N6–H). EIMS: m/z 407 (M⁺, 67), 390 (41), 375 (20), 360 (35), 311 (10), 283 (13), 268 (9), 253 (18), 193 (14), 166 (53), 136 (100), 91 (13), 56 (17%).

1[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-ethanolamine (17)

White amorphous powder; Yield: 0.067 g (86%); Mp = 141–142 °C; R_f = 0.49 (EtOAc: CH₂Cl₂, 2:8); [Found: C (52.43), H (5.77), N (16.09). C₁₉H₂₅N₅O₅S requires C (52.40), H (5.79), N (16.08)]; IR (KBr): ν_{\max} 3514 (O–H str.), 3266 (N–H str.), 2932, 2874 (C–H str.), 1699 (C=O), 1603 (N–H bend.), 1577, 1485 (Ar C=C str.), 1554 (CH₂ bend.), 1393, 1358 (CH₃ bend.), 1323 (S=O asym. str.), 1244 (C–N str.), 1161 (C–O) cm⁻¹; ¹H-NMR (CDCl₃, 500 MHz): δ 0.94 (t, J = 7.4 Hz, 3H, CH₂–CH₂–CH₃), 1.57 (t, J = 6.8 Hz, 3H, O–CH₂–CH₃), 1.80 (m, 2H, CH₂–CH₂–CH₃), 2.84 (dd, J = 7.1 Hz, 7.5 Hz, 2H, CH₂–CH₂–CH₃), 3.01 (m, 2H, NH–CH₂–CH₂–OH), 3.54 (m, 2H, NH–CH₂–CH₂–OH), 4.19 (s, 3H, N1-CH₃), 4.27 (dd, J = 6.8 Hz, 13.8 Hz, 2H, O–CH₂–CH₃), 4.96 (s br, 1H, NH–CH₂–CH₂–OH), 7.09 (d, J = 8.6 Hz, 1H, C-3'H), 7.88 (dd, J = 2.4 Hz, 8.6 Hz, 1H, C-4'H), 8.62 (d, J = 2.4 Hz, 1H, C-6'H), 9.62 (s, 1H, N6–H). EIMS: m/z 435 (M⁺, 79), 420 (36), 406 (58), 363 (10), 312

(73), 282 (90), 268 (13), 253 (23), 238 (18), 193 (15), 166 (78), 136 (77), 98 (100), 73 (10), 56 (14%).

1[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-thioethanol (18)

White amorphous powder; Yield: 0.078 g (88%); Mp = 188–189 °C; R_f = 0.50 (EtOAc: CH₂Cl₂, 2:8); Analysis: [Found: C (50.44), H (5.34), N (12.37). C₁₉H₂₄N₄O₅S₂ requires C (50.43), H (5.35), N (12.38)]; IR (KBr): ν_{\max} 3432 (O–H str), 3325 (N–H str), 2934, 2873 (C–H str.), 1700 (C=O), 1600 (N–H bend.), 1577, 1463 (Ar C=C str.), 1528 (CH₂ bend.), 1393 (CH₃ bend.), 1334 (S=O asym. str.), 1247 (C–N str.), 1160 (C–O) cm^{−1}; ¹H-NMR (CDCl₃, 500 MHz): δ 1.01 (t, J = 7.3 Hz, 3H, CH₂–CH₂–CH₃), 1.63 (t, J = 6.9 Hz, 3H, O–CH₂–CH₃), 1.84 (m, 2H, CH₂–CH₂–CH₃), 2.90 (dd, J = 7.3 Hz, 7.7 Hz, 2H, CH₂–CH₂–CH₃), 2.93 (m, 2H, S–CH₂–CH₂–OH), 3.80 (m, 2H, S–CH₂–CH₂–OH), 4.25 (s, 3H, N1-CH₃), 4.38 (dd, J = 6.9 Hz, 13.6 Hz, 2H, O–CH₂–CH₃), 7.16 (d, J = 8.8 Hz, 1H, C-3'H), 7.96 (dd, J = 2.3 Hz, 8.8 Hz, 1H, C-4'H), 8.96 (d, J = 2.3 Hz, 1H, C-6'H), 10.5 (s, 1H, N6-H). EIMS: m/z 452 (M⁺, 61), 437 (31), 424 (94), 375 (10), 363 (15), 312 (73), 282 (67), 268 (13), 253 (23), 238 (18), 193 (11), 165 (31), 135 (43), 98 (100), 73 (10), 56 (14%).

1[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-nitrophenylhydrazine (19)

White amorphous powder; Yield: 0.083 g (75%); Mp = 211–212 °C; R_f = 0.54 (EtOAc: CH₂Cl₂, 2:8); [Found: C (52.38), H (4.77), N (18.59). C₂₃H₂₅N₇O₆S requires C (52.36), H (4.78), N (18.59)]; IR (KBr): ν_{\max} 3405, 3299 (N–H str.), 2931, 2872 (C–H str.), 1694 (C=O), 1601 (N–H bend.), 1595 (NO₂ str.), 1578, 1465 (Ar C=C str.), 1524 (CH₂ bend.), 1393 (CH₃ bend.), 1346 (S=O asym. str.), 1237 (C–N str.), 1140 (C–O) cm^{−1}; ¹H-NMR (pyridine-*d*₅, 500 MHz): δ 0.96 (t, J = 7.3 Hz, 3H, CH₂–CH₂–CH₃), 1.33 (t, J = 6.8 Hz, 3H, O–CH₂–CH₃), 1.92 (m, 2H, CH₂–CH₂–CH₃), 2.96 (dd, J = 7.0 Hz, 7.6 Hz, 2H, CH₂–CH₂–CH₃), 3.81 (dd, J = 6.8 Hz, 13.8 Hz, 2H, O–CH₂–CH₃), 4.30 (s, 3H, N1-CH₃), 7.08 (d, J = 8.8 Hz, 1H, C-3'H), 7.31 (d, J = 8.9 Hz, 2H, C-2''H/C-6''H), 8.20 (d, J = 8.9 Hz, 2H, C-3''H/C-5''H), 8.35 (dd, J = 2.8 Hz, J = 8.7 Hz, 1H, C-4'H), 8.95 (d, J = 2.8 Hz, 1H, C6'-H), 10.53 (s, 1H, N6-H). EIMS: m/z 527 (M⁺, 21), 482 (9), 391 (15), 375 (10), 363 (15), 312 (73), 282 (100), 268 (13), 253 (20), 238 (21), 193 (16), 165 (25), 135 (35), 98 (80), 73 (11), 56 (18%).

1[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-N-4-mehtylpiperidinyllamine (20)

Yellow semisolid; Yield: 0.095 g (70%); R_f = 0.49 (EtOAc: CH₂Cl₂, 3:7); [Found: C (56.54), H (6.59), N (17.21). C₂₃H₃₂N₆O₄S requires C (56.54), H (6.60), N (17.20)]; IR (KBr): ν_{\max} 3290, 3310 (N–H str.), 2917, 2861 (C–H str.),

1701 (C=O), 1599 (N–H bend.), 1549, 1470 (Ar C=C str.), 1531 (CH₂ bend.), 1385 (CH₃ bend.), 1330 (S=O asym. str.), 1241 (C–N str.), 1158 (C–O) cm^{−1}; ¹H-NMR (CDCl₃, 500 MHz): δ 0.90 (t, J = 7.5 Hz, 3H, CH₂–CH₂–CH₃), 1.22 (m, 4H, C-3''H/C-5''H), 1.49 (t, J = 6.9 Hz, 3H, O–CH₂–CH₃), 1.74 (m, 4H, C-2''H/C-6''H), 1.84 (br s, 1H, C-4''H), 2.24 (m, 2H, CH₂–CH₂–CH₃), 2.80 (dd, J = 7.2 Hz, 7.8 Hz, 2H, CH₂–CH₂–CH₃), 3.22 (br d, J = 9.8 Hz, 2H, NH–CH₂–piperidinyll), 4.14 (s, 3H, N1-CH₃), 4.35 (dd, J = 6.8 Hz, 13.7 Hz, 2H, O–CH₂–CH₃), 7.07 (d, J = 8.7 Hz, 1H, C-3'H), 7.73 (dd, J = 2.1 Hz, J = 8.7 Hz, 1H, C-4'H), 8.52 (d, J = 2.1 Hz, 1H, C-6'H), 10.25 (s, 1H, N6-H). EIMS: m/z 488 (M⁺, 24), 474 (11), 460 (8), 447 (17), 419 (9), 405 (8), 391 (16), 375 (18), 312 (48), 268 (11), 253 (15), 239 (20), 192 (27), 166 (47), 136 (28), 98 (100), 73 (15), 56 (22%).

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